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Acetyl-CoA: L-Homoserine O-Acetyltransferase of the Yeast *Saccharomyces cerevisiae:* Substrate kinetics (Note)

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Acetyl-CoA:L-homoserine O-acetyltransferase [EC 2.3.1.31] has been extracted and partially purified from several microorganisms, as mentioned in a previous paper[1]. In most cases, purification of the enzyme remained incomplete, due to the enzyme's instability and its low contents in the cell. Detailed characterization of the enzyme of *Saccharomyces cerevisiae* has been hindered for the same reasons, although some properties have been investigated after stabilization and partial purification[1]. This paper will deal with the substrate kinetics.

The enzyme was partially purified from a total wet weight of 1,090g of cells of a methionine auxotroph of *S. cerevisiae*, as reported previously[1]. The enzyme activity was determined as described previously[1]. One unit of the enzyme was defined as the amount catalyzing consumption of 1 μmole of acetyl-CoA/min. Protein concentration was determined by the method of Bradford[2] using Coomassie brilliant blue G-250.

The specific activity of the partially purified enzyme preparation was 57.4 units/mg of protein. Reaction velocities were determined, using this preparation, against various concentrations of acetyl-CoA (homoserine) at several fixed concentrations of homoserine (acetyl-CoA). The results are summarized in Fig. 1A and 1B in the form of double reciprocal plots. The parallel lines obtained in both cases suggest that the reaction mechanism of this enzyme is of the "Ping Pong "type[3]. The same mechanism has also been reported for the enzymes of *Brevibacterium flavum*[4] and *Bacillus polymyxa*[5]. The Km values for L-homoserine and acetyl-CoA were calculated to be 1.3 mM and 0.10 mM, respectively, from the intercepts at horizontal axes in both A and B (see the legend to the figure). The Km Value (0.10 mM) for acetyl-CoA, which was determined exactly in this experiment, is considerably higher than the value (0.027 mM) tentatively determined at fixed homoserine concentrations of 2 mM and 5 mM in the previous experiment[1]. The acetyl-CoA value is, however, intermediate between 0.05 mM for *B. flavum*[4] and 0.2 mM for *B. polymyxa*[5]. The Vmax value of the preparation obtained in this experiment was calculated to be approximately 71 units/mg of protein.

Further purification of the partially purified enzyme preparation was examined. A portion of the enzyme preparation (6.5 units) obtained above was applied to a column (1×9 cm) of L-homoserine-immobilized aminohexyl-Sepharose 4B equilibrated with 0.05 M Tris-HCl buffer, pH 7.8, containing 0.25 mM dithiothreitol, 1.0 mM EDTA, and 25% sucrose. The enzyme was eluted with 80 ml of a linear concentration gradient of NaCl from 0 to 500 mM formed in the

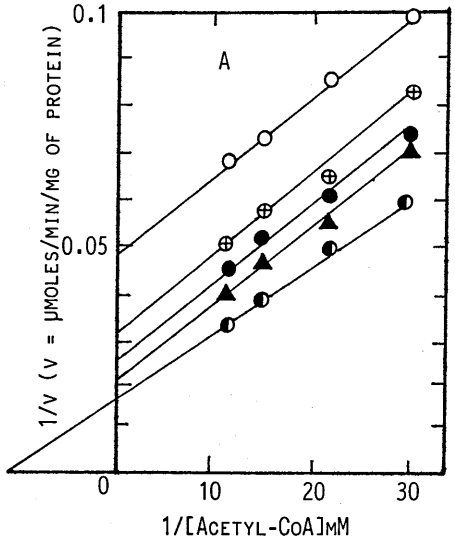

Fig. 1 (A): Double reciprocal plot of reaction velocities against concentrations of acetyl-CoA. A portion of an enzyme preparation, containing 0.093μg of protein, was employed in a reaction mixture containing 5,5'-dithiobis (2-nitrobenzoic acid). Concentrations of L-homoserine were 0.5 mM (○), 1.0 mM (⊕), 1.5 mM (●), and 2.0 mM (▲).

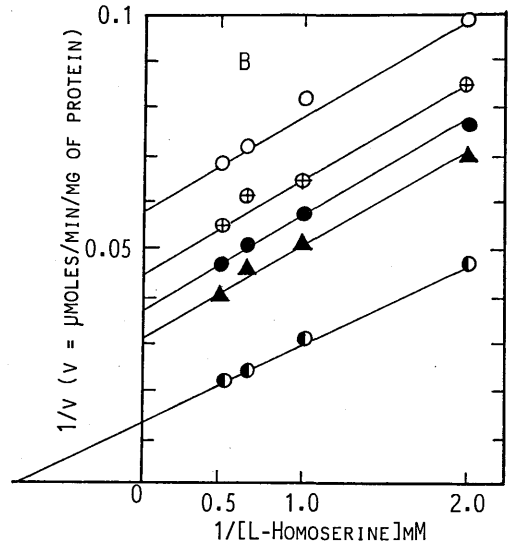

Fig. 1 (B): Double reciprocal plot of reaction velocities against concentrations of L-homoserine. Experimental conditions were the same as in (A). Concentrations of acetyl-CoA were 0.033 mM (○), 0.050 mM (⊕), 0.067 mM (●), and 0.086 mM (▲).

The straight lines (Ⓘ) shown in both (A) and (B) are the results of secondary plots of the intercepts at vertical axes in (B) and (A), respectively, against reciprocals of concentrations of the two substrates.

same buffer, and the eluate was fractionated by 1.85 ml. Polyacrylamide gel electrophoresis of the active fractions still showed two major protein components (data not shown).

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